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A Proposed 1st WHO International Genetic Reference Panel for the quantitation of *BCR-ABL* translocation by RQ-PCR, NIBSC code 09/138

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Summary

Ten laboratories participated in an international collaborative study to assess the suitability of a panel of freeze-dried cells for the quantitation of *BCR-ABL* translocation. The code numbers of the individual materials were 08/192 (BCR-ABL 1), 08/194 (BCR-ABL 2), 08/196 (BCR-ABL 3) and 08/198 (BCR-ABL 4). The aim of the study was to assign values to the new materials using the existing international scale. Conclusions from this study indicated that all of the materials were suitable to be used as reference materials for the quantitation of *BCR-ABL* translocation using real time quantitative PCR (RQ-PCR). All of the participating laboratories agreed with the recommendation.

Introduction

Chronic Myelogenous Leukemia (CML) represents about 15-20% of all cases of adult leukemia and Acute Lymphoblastic Leukemia (ALL) accounts for approximately 80% of all childhood leukemia cases. Nearly all cases of CML and a minority of cases of ALL are caused by a chromosome translocation t(9;22)(q34;q11), known as the Philadelphia chromosome which fuses 2 genes: *BCR* & *ABL*. The *BCR-ABL* fusion acts as an oncogene and promotes genomic instability.

Reverse-transcription real-time quantitative PCR (RQ-PCR) is routinely used to quantify levels of *BCR-ABL* mRNA transcripts in peripheral blood and bone marrow samples from chronic myeloid leukaemia (CML) patients. The technique can determine accurately the response to treatment and is particularly valuable for patients who have achieved complete chromosomal remission. Despite efforts to establish standardised protocols for *BCR-ABL* fusion transcript quantitation[1] there is still substantial variation in the way in which RQ-PCR for *BCR-ABL* is carried out and how results are reported in different laboratories worldwide[2]. In particular, the use of different control genes for normalisation of results means that there are several different units of measurement worldwide, e.g. *BCR-ABL / ABL*; *BCR-ABL / BCR*; *BCR-ABL / GUSB, BCR-ABL / G6PD, BCR-ABL / β2M* etc.

The IRIS clinical trial involved 1106 patients in 16 countries and established the current standard of care in CML. The trial employed RQ-PCR molecular monitoring of patient response but found that there were significant differences in *BCR-ABL* values obtained at different centres which prompted the need for an urgent alignment of the respective results. In the absence of any independent reference materials, the decision was made that each centre would measure the level of disease in a common set of 30 pre-treatment samples, and that patient results would be normalised to this standardised baseline. Reanalysis of the data showed improved comparability of results between laboratories[3].

Following on from the IRIS trial, the CML meeting at the National Institutes of Health in Bethesda in October 2005 made several recommendations for the harmonisation of RQ-PCR for *BCR-ABL* including the use of one of three control genes (*ABL*, *BCR* or *GUSB*) [4,5]. Most importantly, a new international scale (IS) for *BCR-ABL* RQ-PCR measurements was proposed which is anchored to two key levels used in the IRIS study, namely a standardised baseline

defined as 100% *BCR-ABL* (IS), and major molecular response (3 log reduction relative to the standardised baseline) defined as 0.1% *BCR-ABL* (IS). The strength of the IS is that (i) laboratories can continue to use their existing assay conditions (provided their assay is linear on analysis of test samples), and (ii) that they can continue to express results according to local preferences in addition to expressing results on the IS. The concept of the IS is similar to established procedures for other quantitative assays, for example the International Normalised Ratio (INR) for prothrombin time.

The original standards used for the IRIS trial are no longer available, however traceability to the IRIS scale is provided by the extensive quality control data generated by the Adelaide laboratory (Division of Molecular Pathology, Institute of Medical and Veterinary Science, Adelaide, Australia) over a period of several years[6]. Establishment of the IS therefore requires the alignment of local test results either directly or indirectly with those obtained in Adelaide. Currently, this can be achieved by exchange of a series of patient samples with either the Adelaide or Mannheim (Wissenschaftliches Labor, III. Medizinische Klinik, Mannheim, Germany) international reference laboratories to derive a laboratory-specific conversion factor to the IS[6]. Although this system works well, it is very laborious and consequently only open to a limited number of laboratories at any given time. The availability of internationally accredited reference reagents should in principle help to make the IS more accessible, as well as providing a more robust framework for the scale itself. This will allow greater comparability between laboratories and lead to the standardisation of patient assessment.

Ideally, the formulation for reference reagents should be as close as possible to the usual analyte, should cover the entire analytical process and should be applicable to methods in use throughout the world. However it is essential that the formulation is stable over a period of several years and that it is physically possible to produce batches of sufficient size to satisfy demand over a similar period of time. It has been shown previously that good quality RNA can be extracted from freeze dried K562 cells[7] and therefore one possible solution is the use of freeze dried cell line mixtures. This strategy was presented and agreed upon at a meeting of the International BCR-ABL standardization group, an informal network which meets biannually at the annual American Society of Hematology and European Hematology Association meeting and is attended by representatives from public and private testing laboratories throughout the world.

Aims of study

The aim of this collaborative study was to produce and perform an assessment of the use of freeze dried cell line mixtures as universal reference materials in support of the IS. In earlier studies we identified cell lines in which the relative ratio of the three recommended control genes – *BCR, ABL* and *GUSB* – was similar to that seen in normal leucocytes. Twenty-five haemopoietic cell lines (ACC42, Jurkat, JVM2, Loucy, MOLT3, NALM76, PEER, REH, RS4-11, T-ALL1, THP1, HeLa, MV4-11, Karpas, ML-1, ML-2, 1E8, ALLPo, NB4, KG1, KG1a, HL60, Kasumi1, NOMO1, SKM1) and one colorectal adenocarcinoma cell line (Caco2) were tested in up to four different centres and KG1 and HL60 were identified as the best candidates to take forward for detailed evaluation. Following further field trial studies[7] the HL60 cell line was chosen as the source of control genes, because the relative expression levels of the control genes

was closest to those found in normal leucocytes, and K562 cells were used as the source of the *BCR-ABL* translocation.

This report describes the preparation of four reference materials and their evaluation in an international field trial that involved 10 laboratories (6 EU, 1 Canada, 3 Asia/Australasia) using 4 different protocols and 8 different RQ-PCR platforms. The aims of this field trial were to: i) test four different dilution levels of K562 diluted in HL60 by measuring absolute copy numbers of *BCR-ABL* transcripts, absolute copy numbers of control gene transcripts and the *BCR-ABL / control gene* ratios and ii) assign fixed *% BCR-ABL / control gene* values, linked to the IS, to each material.

Participants

Eleven laboratories were invited to take part in the study. The laboratories were chosen because they all have a validated and stable conversion factor (CF), assigned by one of the two international reference laboratories, which allows them to convert results generated with their own local method to results expressed using the international scale (IS). Ten laboratories agreed to participate (Appendix 1) and all returned results. Nine different countries were represented. Each laboratory has been assigned a code number which does not reflect the order of listing in Appendix 1.

Materials

HL60 cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) and K562 cells were obtained from the Hammersmith Hospital, London, UK. Specific permission was obtained for their use and subsequent distribution as a reference material. Both cell lines were tested for HIV, HBV, HCV, CMV, EBV, HTLV-I/II, HHV-8 and mycoplasma by PCR, and none were found.

Both cell lines were grown by the European Collection of Cell Cultures (ECACC) in RPMI 1640 medium supplemented with 10% fetal bovine serum (Sigma Aldrich). Four dilutions of K562 in HL60 were made that corresponded approximately to (BCR-ABL 4; NIBSC material code 08/198), 1% (BCR-ABL 3; 08/196), 0.1% (BCR-ABL 2; 08/194) and 0.01% (BCR-ABL 1; 08/192) *BCR-ABL / ABL*. Cell suspensions in ice cold 2x phosphate buffered saline (PBS) were transferred to the National Institute for Biological Standards and Control (NIBSC) on ice. Cell suspensions were stored at 4ºC overnight with gentle stirring. The following day 3ml glass ampoules were filled with 1.5 x 10^6 cells (0.5ml) for freeze drying. No other stabilisers or bulking agents were added. The drying process took 65-67 hours and approximately 3500 vials of each material were produced. Table 1 shows the product summary for the four materials.

Table 1. Product summaries of the four candidate materials

NIBSC Code	**08/192** **BCR-ABL 1**	**08/194** **BCR-ABL 2**	**08/196** **BCR-ABL 3**	**08/198** **BCR-ABL 4**
Date filled	04.09.08.	04.09.08.	04.09.08.	04.09.08.
Coefficient of variation of the fill (%) (n=132-144)	0.19	0.19	0.28	0.22
Residual moisture after lyophilisation (%) (n=12)	0.4701	0.4208	0.4833	0.4760
Mean dry weight (g) (n=6)	0.0115	0.0115	0.0114	0.0112
Mean residual oxygen% (n=12)	0.20	0.28	0.29	0.43
No of ampoules available	3614	3467	3389	3558
Presentation	Sealed, glass 3mL DIN ampoules			
Excipient	2X phosphate buffered saline			
Address of facility where material was processed	NIBSC, Potters Bar, Herts, UK			
Present custodian	NIBSC, Potters Bar, Herts, UK			
Storage temperature	-20°C			

This standard is intended to be used in the *in vitro* diagnostics field and it relates to BS EN ISO 17511:2003 Section 5.5.

Routine microbiology testing of the freeze-dried materials showed contamination with Staphylococcus haemolyticus, a common human skin commensal which is classified in Hazard Group 2. Experiments carried out by a microbiologist at NIBSC showed that the organism was completely killed after exposure to Trizol (30-60% Phenol) which is the first step of the RNA extraction process.

Stability studies

Accelerated degradation studies were carried out using reverse transcription real-time quantitative PCR (RQ-PCR) using samples stored at elevated temperatures compared with others stored at -150°C. Two sets of data were derived for each material; the absolute copy number of the relevant gene transcripts (*BCR-ABL, ABL, BCR* & *GUSB*) and the ratio between *BCR-ABL* and the three control gene transcripts. Data obtained after six months of storage indicates that in all four preparations there has been a slight reduction in the copy number of all genes when stored at +56°C and +45°C (Figure 1) and that the BCR-ABL / *control gene* transcript ratio, which is the critical unit of MRD measurement, has begun to drop at +56°C (Table 2). These preliminary results show therefore that the materials are still acceptable for analysis if stored at +45°C or below for 6 months. However, further accelerated degradation studies at elevated temperatures and a real-time degradation study of the -20°C samples against the -150°C will be

carried out to monitor the stability of the panel.

Figure 1. Accelerated degradation studies after storage of ampoules at elevated temperatures for 6 months. Mean of control gene transcript levels in all 4 materials

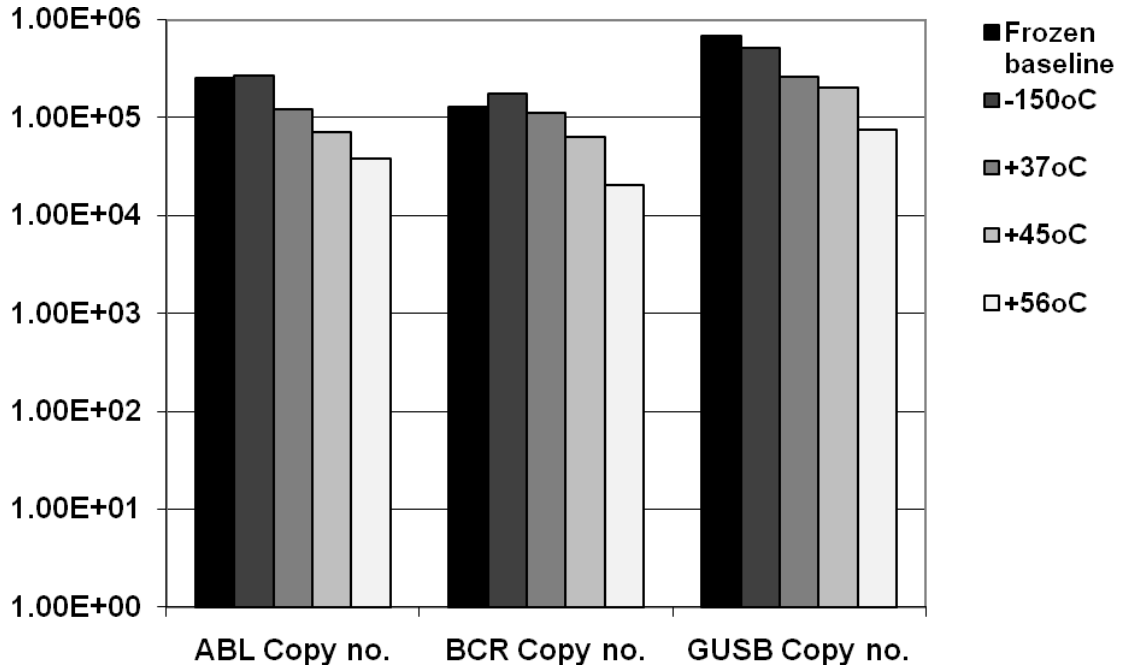


Table 2. %BCR-ABL / ABL (IS) after storage of ampoules at elevated temperatures for 6 months

	Frozen baseline	**-150°C**	**37°C**	**45°C**	**56°C**
BCR-ABL 4 08/198	10.3191	11.7196	9.4173	9.7961	7.1719
BCR-ABL 3 08/196	1.1506	1.3786	1.0808	1.3549	0.8713
BCR-ABL 2 08/194	0.1175	0.1131	0.1089	0.1181	0.0812
BCR-ABL 1 08/192	0.0143	0.0154	0.0112	0.0146	0.0081

Homogeneity testing

To assess the homogeneity of the material distributed into the glass ampoules, 17 vials (picked at random) of each reference material (BCR-ABL 1 to 4) were tested in triplicate in the same RQ-PCR run. In order to make a comparison with the intra-assay variability, two similar samples (non-lyophilised BCR-ABL 1 and BCR-ABL 4) were each tested multiple times (17 triplicate reactions). The coefficient of variation (CV) of *%BCR-ABL / control gene* for each reference material tested in triplicate in the same run is shown in table 3.

Table 3. Coefficient of variation (%) for triplicate analysis of 17 vials of freeze dried cells of each reference material and 2 samples of non-lyophilised cell line mixtures each tested 17 times

17 ampoules tested	**%CV** ***BCR-ABL / ABL***	**%CV** ***BCR-ABL / BCR***	**%CV** ***BCR-ABL / GUSB***
BCR-ABL 1 08/192	23.31	21.10	18.92
BCR-ABL 2 08/194	12.84	15.74	19.57
BCR-ABL 3 08/196	9.07	13.14	13.21
BCR-ABL 4 08/198	14.23	11.61	16.02
1 sample tested 17 times			
BCR-ABL 1 frozen	10.31	14.52	14.52
BCR-ABL 4 frozen	13.31	19.20	13.93

The results suggest that the variability between ampoules of the candidate materials is no greater than the variability of the assay itself. For BCR and GUSB control genes there was no significant difference in the variabilities shown above (p=0.314 and p=0.538 respectively). For the ABL control gene there was a significant difference in variabilities (p=0.009). This is due to the higher variability observed for 08/192 (CV 23.31%) and no significant difference is found when this data is removed (p=0.299).

Study design

The aim of the study was to evaluate a panel of four freeze-dried preparations containing the same two cell lines in different proportions, in order to assign fixed % BCR-ABL / control gene values to each material.

The performance of the freeze dried cells was assessed by an international collaborative study carried out between January and March 2009. The study involved ten laboratories using four different protocols and eight different RQ-PCR platforms.
Freeze dried cells in glass ampoules were distributed to laboratories by courier at ambient temperature. Twelve glass ampoules were supplied in three bags labelled Batch 1, Batch 2 and Batch 3, thus allowing the same procedure to be carried out on three separate occasions. Each bag contained 4 ampoules:

BCR-ABL 1 08/192	approx 0.01% BCR/ABL
BCR-ABL 2 08/194	approx 0.1% BCR/ABL
BCR-ABL 3 08/196	approx 1.0% BCR/ABL
BCR-ABL 4 08/198	approx 10% BCR/ABL

RNA was extracted from each batch on different days using the entire lysate for each RNA extraction. Two cDNA synthesis reactions were performed for each of the extracted RNA samples from each batch using the established methods of the participating laboratories. The cDNA synthesis reactions were performed on different days, giving a total of 24 cDNA samples.

RQ-PCR for *BCR-ABL* and the control gene(s) was performed on each set of 4 cDNA samples for each batch in separate quantitative runs.

Methods

Three control genes were analysed in this study: *ABL*, *BCR* and *GUSB*. Although some laboratories returned results for control genes where they did not have a validated conversion factor (CF), these were not used in the calculation of the final values assigned to each material. Only data from laboratories with validated CFs (Table 4) were included in the calculation.

Table 4. Laboratories with validated conversion factors for each control gene

Lab ID	ABL	BCR	GUSB
Lab 1		✓	
Lab 2			✓
Lab 3	✓		✓
Lab 4		✓	
Lab 5	✓		
Lab 6	✓		
Lab 7	✓		
Lab 8		✓	
Lab 9	✓		✓
Lab 10	✓		

Samples for *BCR-ABL* and the control gene(s) were analysed using the established methods of the participating laboratories (which are representative of the main methodological variants that are in use worldwide);

- **RQ-PCR machines.** Eight different types of RQ-PCR machines were used: ABI 7500 (n=3), ABI 7000 (n=1), ABI 7700 (n=1), ABI 7900 (n=1), ABI 7300 (n=1), Corbett RotorGene 6000 (n=1), Roche LightCycler 1.5 (n=1), Roche LightCycler 480 (n=1)

- **RNA extraction methods.** Two RNA extraction methods were used; 8 labs used Trizol (Invitrogen) and 2 used QIAGEN extraction kits.

- **RT-PCR methods**. Six of the participants cited Gabert et al. (2003)[1] as one of the references used as their RQ-PCR protocol. Other references cited were Branford et al. (1999)[8] and Emig et al (1999)[9]. One laboratory used an 'in house' developed assay. All labs used random hexamers for reverse transcription.

The following data were recorded; date of RNA extraction, total µg of RNA, A_{260} / A_{280}, A_{260} / A_{230}, date of cDNA synthesis, final volume of cDNA reaction, volume of cDNA added to RQ-PCR, date of RQ-PCR, slope and gradient of standard curve, *BCR-ABL* transcript value (Ct value and copy number) control gene(s) transcript value (Ct value and copy number), *BCR-ABL* /

control gene(s) (%) before conversion to IS, *BCR-ABL* / *control gene(s)* (%) converted to IS.

Results

All participants returned results and no significant problems were reported. Lab 1 reported a low RNA yield but their *BCR-ABL* / *control gene* transcript values were as expected. Lab 8 had to repeat some tests in accordance with their local best practice guidelines for investigating clinical samples, but again their *BCR-ABL* / *control gene* transcript values were as expected. The mean amount of RNA extracted by individual laboratories from each material is shown in table 5 below. Most cDNA synthesis reactions require only 2µg RNA.

Table 5. Amount of RNA extracted from materials. n = 30 (3 ampoules in 10 laboratories)

Material	Mean µg RNA
BCR-ABL 1 08/192	14.86
BCR-ABL 2 08/194	14.34
BCR-ABL 3 08/196	14.16
BCR-ABL 4 08/198	17.42

Laboratories tested cDNA in their quantitative PCR and the resulting cycle threshold (Ct) values were converted to gene transcript copy number using standard curves. Each of the four materials was tested 6 times (extraction on 3 separate days, tested in duplicate). For each of the four materials the transcript copy numbers for *BCR-ABL* and the three control genes were used to calculate *%BCR-ABL* / ABL, *%BCR-ABL* / *BCR* and *%BCR-ABL* / *GUSB*. The arithmetic means of these 6 reactions in each laboratory are shown in tables 6-8 along with the corresponding % coefficient of variation (CV). The mean % values for each material were then converted to the IS using a conversion factor previously supplied by the reference laboratory.

The harmonizing effect of the use of individual conversion factors for each laboratory can be seen in figure 2. The data from each laboratory in this study is shown before conversion to IS in the upper graph, and after conversion in the lower one.

Table 6. Mean values for ***%BCR-ABL / ABL*** for each laboratory, a) before and b) after conversion to IS

a)

Lab	BCR-ABL 1 08/192		BCR-ABL 2 08/194		BCR-ABL 3 08/196		BCR-ABL 4 08/198	
	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)
3	0.0086	42.72	0.0976	19.36	1.1863	17.85	13.9092	9.26
5	0.0161	74.36	0.1285	10.99	1.3316	12.67	11.8597	15.77
6	0.0617	15.80	0.3410	6.39	3.0366	2.42	25.3037	2.82
7	0.0715	32.44	0.8270	26.48	8.6209	9.97	66.4860	12.02
9	0.0120	46.64	0.1983	23.86	2.2190	16.00	23.8594	29.10
10	0.0238	40.10	0.2185	37.41	2.2352	22.35	20.7830	14.73
Overall mean	0.0323		0.3018		3.1049		27.0335	
Between-lab CV %	84.37		89.73		89.72		74.19	

b)

Lab	Mean IS			
	BCR-ABL 1 08/192	BCR-ABL 2 08/194	BCR-ABL 3 08/196	BCR-ABL 4 08/198
3	0.0076	0.0857	1.0416	12.2123
5	0.0137	0.1093	1.1323	10.0843
6	0.0142	0.0784	0.6984	5.8198
7	0.0165	0.1902	1.9828	15.2918
9	0.0041	0.0676	0.7567	8.1360
10	0.0148	0.1360	1.3914	12.9374
Overall mean	0.0118	0.1112	1.1672	10.7469
Between-lab CV %	41.00	41.18	40.56	32.00

Table 7. Mean values for ***%BCR-ABL / BCR*** for each laboratory, a) before and b) after conversion to IS

a)

Lab	BCR-ABL 1 08/192		BCR-ABL 2 08/194		BCR-ABL 3 08/196		BCR-ABL 4 08/198	
	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)
1	0.0016	67.22	0.0115	25.55	0.0876	25.15	1.0354	13.16
4	0.0167	30.50	0.1799	31.82	1.8261	31.73	17.8138	36.70
8	0.0167	35.55	0.1431	25.23	1.4058	20.72	12.4605	49.56
Overall mean	0.0117		0.1115		1.1065		10.4366	
Between-lab CV %	74.73		79.40		81.98		82.12	

b)

Lab	Mean IS			
	BCR-ABL 1 08/192	BCR-ABL 2 08/194	BCR-ABL 3 08/196	BCR-ABL 4 08/198
1	0.0164	0.1168	0.8936	10.5613
4	0.0214	0.2303	2.3374	22.8017
8	0.0208	0.1789	1.7573	15.5756
Overall mean	0.0195	0.1753	1.6627	16.3129
Between-lab CV %	13.98	32.41	43.69	37.72

Table 8. Mean values for ***%BCR-ABL / GUSB*** for each laboratory, a) before and b) after conversion to IS

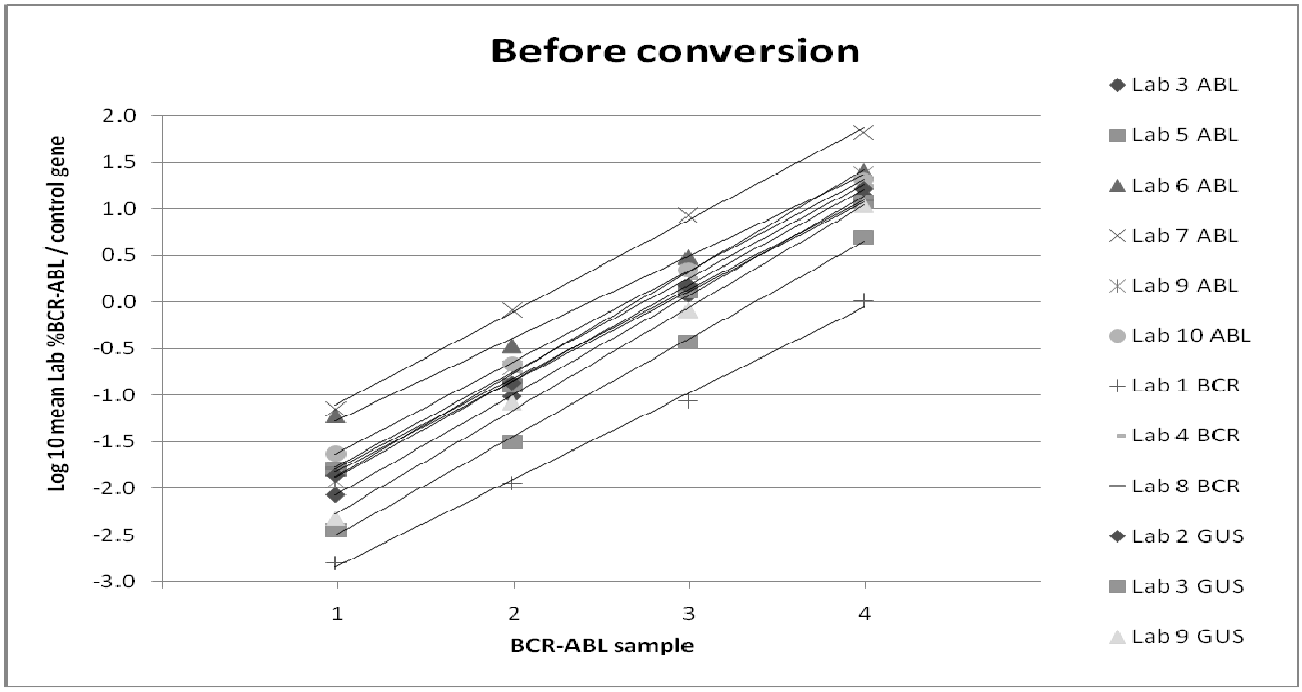
a)

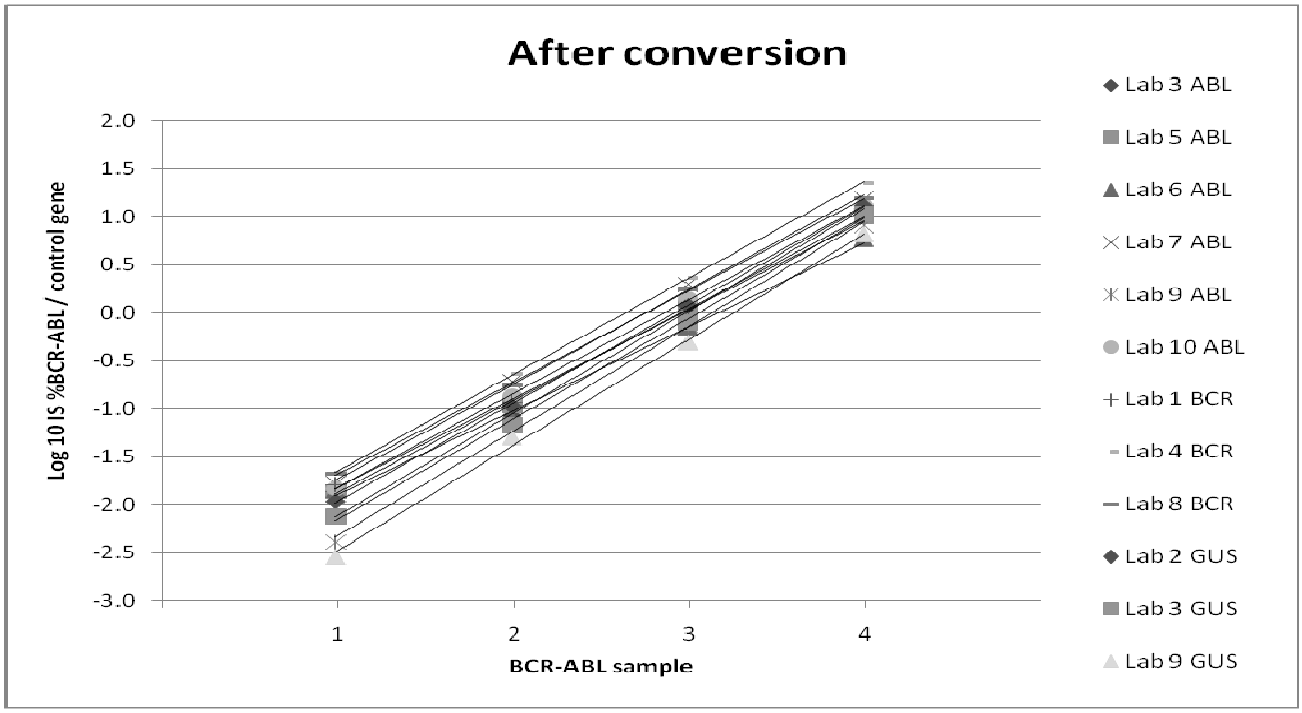
Lab	BCR-ABL 1 08/192		BCR-ABL 2 08/194		BCR-ABL 3 08/196		BCR-ABL 4 08/198	
	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)	Mean	Intra-lab CV (%)
2	0.0138	31.48	0.1354	16.24	1.5174	10.97	16.6688	18.33
3	0.0036	70.76	0.0315	21.47	0.3740	26.24	4.9804	12.06
9	0.0047	52.19	0.0837	42.52	0.8164	29.12	10.9180	55.93
Overall mean	0.0074		0.0835		0.9026		10.8557	
Between-lab CV %	76.00		62.19		63.88		53.84	

b)

Lab	Mean IS			
	BCR-ABL 1 08/192	**BCR-ABL 2 08/194**	**BCR-ABL 3 08/196**	**BCR-ABL 4 08/198**
2	0.0109	0.1069	1.1981	13.1616
3	0.0076	0.0675	0.8004	10.6580
9	0.0028	0.0502	0.4898	6.5508
Overall mean	0.0071	0.0749	0.8295	10.1235
Between -lab CV %	57.37	38.81	42.81	32.97

Figure 2. Log transformed data from all labs, before and after conversion to the IS





The mean *%BCR-ABL / control gene* transcript values obtained following conversion to the international scale are shown in table 9. We propose that these international scale values should be assigned to this batch of material.

Table 9. Proposed values for *%BCR-ABL / control gene*, for all materials

	% BCR-ABL / ABL	*% BCR-ABL / BCR*	*% BCR-ABL / GUSB*
BCR-ABL 1 08/192	0.0118	0.0195	0.0071
BCR-ABL 2 08/194	0.1112	0.1753	0.0749
BCR-ABL 3 08/196	1.1672	1.6627	0.8295
BCR-ABL 4 08/198	10.7469	16.3129	10.1235

Participant Responses

All laboratories that participated in the collaborative studies were sent a copy of the final report. Approval was sought for the proposal that these materials should be proposed to WHO as the 1st International Genetic Reference Panel for the quantitation of *BCR-ABL* translocation, and that the materials should have the values in table 9. All laboratories agreed with the proposal.

Discussion

The advent of effective targeted therapy for CML in the late 1990s prompted the need for accurate measurement of the amount of the abnormal clone remaining in the patient. Monitoring patient response can be achieved by three principal approaches: (i) haematological assessment of leukocyte counts and bone marrow morphology (ii) cytogenetic analysis to determine the proportion of Philadelphia chromosome positive bone marrow cells and (iii) determination of the amount of BCR-ABL mRNA by RQ-PCR. All three techniques are in routine use, but RQ-PCR is by far the most sensitive and is the only technique that can gauge two important therapeutic milestones, major molecular response and complete molecular remission. Furthermore, RQ-PCR is routinely performed on peripheral blood samples and is therefore less invasive than techniques that require bone marrow aspirates.

The amount of BCR-ABL mRNA is measured by comparison with the level of a control gene transcript (unaffected), which acts as an internal control for sample quality and quantity. However, this is the limit of conformity and several different control genes, assay methods and reporting strategies are in use by different groups around the world. There was a clear need for standardisation of measurement and at the same time the relationship between BCR-ABL levels and clinical outcome needed to be established. The IRIS trial established a standard baseline for measurement (100% BCR-ABL on the ‘international scale’) and a major molecular response (MMR, good response to therapy) was defined as a 3 log reduction in the amount of BCR-ABL (0.1% BCR-ABL on the ‘International Scale’). However, the samples used to define these values were quickly exhausted and traceability relied on the internal QC data of one laboratory in Adelaide. Other laboratories wishing to align their results with the International Scale could do so by exchanging samples with the Adelaide laboratory and by this process conversion factors were established for several laboratories. Subsequently one more laboratory in Mannheim has

become able to derive conversion factors and although this system works well, it is very laborious and consequently only open to a limited number of laboratories at any given time. The current study has assigned *BCR-ABL* / *control gene* values for four different freeze-dried materials, each containing different amounts of *BCR-ABL*. Alignment of the current materials with the pre-existing 'international scale' is vital to allow consistent and comparable quantitative data to be obtained in all countries. The values have been assigned by a small number of expert laboratories who have been able to show that their conversion factor, which allows them to convert their local results to the international scale, is stable over time. CML occurs in all countries and quantitative measurement of *BCR-ABL* is carried out in many of these. In order to conserve the supply of these materials (approx. 3300 panels) we propose to limit supply to national reference laboratories and manufacturers who will use the materials to calibrate secondary standards.

Conclusions and Proposal

The results of this international multi-centre study show that the materials contained in Panel 09/138 are suitable for use in the quantitation of *BCR-ABL* and it is proposed that the above materials be established as the WHO 1st International Genetic Reference Panel for the quantitation of *BCR-ABL* translocation and that the materials should be assigned the values listed in table 9.

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Appendix I. List of Participants

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Appendix 2. Proposed Instructions For Use

Proposed 1st WHO International Genetic Reference Panel for the quantitation of *BCR-ABL* translocation, NIBSC code 09/138
Instructions for use
(Version 1.0, Dated 24/06/2009)

1. INTENDED USE

The panel comprises four individually coded ampoules, each containing freeze-dried cells. Each ampoule has a different defined value for %BCR-ABL/control gene and they are intended to be used for calibrating secondary standards. This panel was established in 2009 as the 1st WHO International Genetic Reference Panel for the quantitation of *BCR-ABL* translocation, NIBSC code 09/138 by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization (WHO). These materials should not be put to any other use.

2. CAUTION

This preparation is not for administration to humans.

The preparations contain material of human origin. They have been tested and found to be negative for HIV, HBV, HCV, CMV, EBV, HTLV-I/II, HHV-8 and mycoplasma by PCR.
Routine microbiology testing of the free-dried materials showed contamination with Staphylococcus haemolyticus, which is classified in Hazard Group 2. Experiments carried out at NIBSC showed that the organism was completely killed after exposure to Trizol (30-60% Phenol) which is the first step of the RNA extraction process.
As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The panel was tested in an international collaborative study involving 10 laboratories and the following mean *%BCR-ABL* / control gene values were obtained following conversion to the international scale (IS);

Code no.	%BCR-ABL / ABL	%BCR-ABL / BCR	%BCR-ABL / GUSB
08/192	**0.0118**	**0.0195**	**0.0071**
08/194	**0.1112**	**0.1753**	**0.0749**
08/196	**1.1672**	**1.6627**	**0.8295**
08/198	**10.7469**	**16.3129**	**10.1235**

4. CONTENTS

Country of origin of biological material: Germany & United Kingdom.

The ampoules contain freeze-dried K562 cells (expressing the *BCR-ABL* translocation b3a2) and HL60 cells (*BCR-ABL* negative) in varying proportions. The total number of cells per ampoule is 1.5×10^6. The cells were suspended in 2x PBS before freeze-drying.

5. STORAGE

Store all unopened ampoules of the freeze-dried preparations at -20°C or below.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.
Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.
Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

a. Open ampoules as described in section 6. above.
b. Reconstitute freeze-dried material at room temperature with 1mL Trizol or 600 µL RLT (Qiagen).
c. Ensure all cells are lysed by repeated aspiration with a pipette tip or a needle
d. Transfer the entire contents to nuclease-free tubes.
e. Extract RNA using local method.

8. STABILITY

NIBSC follows the policy of WHO with respect to its reference materials. It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Accelerated degradation experiments indicate that the freeze-dried materials in ampoules are stable after incubation at +45° for at least 6 months.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES N/A

10. ACKNOWLEDGEMENTS

We would like to thank the German Collection of Microorganisms and Cell Cultures (DSMZ), the Hammersmith Hospital, London and the UK National Genetics Reference Laboratory (Wessex) for supplying materials and assistance with the collaborative study.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.ac.uk
WHO Biological Standards: Http://www.who.int/biologicals/en/
Derivation of International Units:
Http://www.nibsc.ac.uk/products/faq.asp
Ordering standards from NIBSC:
Http://www.nibsc.ac.uk/products/faq.asp
NIBSC Terms & Conditions: Http://www.nibsc.ac.uk/terms.html

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.ac.uk

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Physical and Chemical properties	
Physical appearance: Freeze-dried solid	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains material of human origin	
Toxicological properties	
Effects of inhalation: Not established, avoid inhalation	
Effects of ingestion: Not established, avoid ingestion	
Effects of skin absorption: Not established, avoid skin contact	
Suggested First Aid	
Inhalation: Seek medical advice	
Ingestion: Seek medical advice	
Contact with eyes: Wash with copious amounts of water. Seek medical advice	
Contact with skin: Wash thoroughly with water	
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

It is the responsibility of the Recipient to determine the appropriateness of the standards or reference materials supplied by the Institute to the Recipient ("the Goods") for the proposed application and ensure that it has the necessary technical skills to determine that they are appropriate. Results obtained from the Goods are likely to be dependent on conditions of use by the Recipient and the variability of materials beyond the control of the Institute.

All warranties are excluded to the fullest extent permitted by law, including without limitation that the Goods are free from infectious agents or that the supply of Goods will not infringe any rights of any third party.

The Institute shall not be liable to the Recipient for any economic loss whether direct or indirect, which arise in connection with this agreement.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom

* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.

Net weight of each ampoule: 0.0114g

Toxicity Statement: Non-toxic

Veterinary certificate or other statement if applicable**.**

Attached: No